

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph at page 4, lines 1-10, with the following rewritten paragraph:

-- Animal venoms have ~~preoved~~proved to be a valuable resource of unique and novel chemical compounds that have shown a wide variety of biological activities. Furthermore, advancements in synthetic organic chemistry have made it possible to synthesize not only newly identified natural products, but also structural analogs and semi-synthetic derivatives. Even with all the advances in synthetic organic chemistry, however, much time and effort is saved by using a model compound established through the elucidation of a novel natural product. --.

Please replace the paragraph at page 5, lines 6-12, with the following rewritten paragraph:

-- Natural venoms are metabolic products not directly essential for the life of producer organism, although they are essential for both predatory and defense needs. Venom constituents can act in many fronts on the victim's tissues such as ionic channels in excitable tissues and specific receptors, like enzymes, for example, leading to ~~disturbs~~disturbances in muscular, cardiovascular or respiratory systems. --.

Please replace the paragraph at page 5, lines 16-27, with the following rewritten paragraph:

-- The most studied and representative classes of bioactive compounds found in scorpion venoms are related to small proteins, i.e., less than 10 kDa, which bind on selective ionic channels of both invertebrates and vertebrates nervous systems. These proteins are able to block

or modulate kinetics of Na^+ , K^+ , Ca^{2+} and Cl^- selective-channels, leading the inflicted victim to a massive neurointoxication and sometimes death. Due to their selectively, scorpion toxins are classified accordingly: 1) to their toxicological spectrum; 2) to their binding sites on ionic selective channels and 3) to their structural family. --.

Please replace the paragraph at page 7, lines 10-22, with the following rewritten paragraph:

-- Peptides that form a structural family were purified from the venom of Brazilian scorpion *Tityus serrulatus*. Structurally, this family is identifiable by the absence of Cys residues and therefore with no disulfide bonds. Moreover, mature peptides from this family ranges from 2500 to 3000 Da and ~~hashave~~ have typical amino acid signatures (Pro-Pro-Ala or Pro-Pro) at their carboxi-terminal extremity. Pharmacologically, these peptides act as bradykinin-potentiating peptides, and therefore, can be used as hypotensive agents. These, structural and pharmacological features; classify members of this peptide family as serious candidates to be used as pharmaceutical drugs in the treatment of hypertension. --.

Please replace the sub-heading on page 7, line 24 with the following sub-heading:

-- A- Definitions --.

Please replace the paragraph at page 8, lines 9-27, with the following rewritten paragraph:

-- **Scorpion Venom:** A secretion produced by the venom gland located at the scorpion telson. The venom constituents, mainly proteins, are produced by the gland cells and secreted into the glandular lumen. It is used in both predatory and defense senses being toxic to many

animals, for example, from insects to humans. Methods of venoms extraction consist on electrical or manual milking, in which the gland content is expelled and collected for further preparations. Alternatively, the whole telson or gland is "~~erashed~~" "crushed" in an organic or inorganic medium and the proteins purified from the whole telson or gland extract. Crude venom or whole venom are related to the complete milked solution without any further preparation such as lyophilization or any separation step. Soluble fraction of venom is related to the venom, preferably lyophilized venom, that was resuspended on a solution, preferably a low ionic strength buffer and submitted to centrifugation to separate the soluble fraction from the solid fraction. --

Please replace the paragraph at page 9, lines 1-4, with the following rewritten paragraph:

-- **Peptide:** A protein with low molecular mass. Although there is not a real boundary between protein and peptide, peptides are considered, in a practical sense, as proteins smaller than 10000 Da. --.

Please replace the paragraph at page 9, lines 5-18, with the following rewritten paragraph:

-- **Liquid chromatography:** A molecular separation method consisting on a mobile phase, a solvent or a buffer, for example, passed throughout a fixed phase with particular physico-chemical properties. Molecules samples are loaded onto a cylindrical tube filled with the fixed phase and are eluted by the mobile phase being separated by means of the physico-chemical properties related to: 1) the molecules themselves; 2) the fixed phase and 3) the mobile phase. Conventional, Fast Performance Liquid Chromatography (FPLC) or High Performance

(or Pressure) Liquid Chromatography (HPLC) are related to the apparatus used. Size-exclusion (or molecular filtration), Ionic (Cationic or Anionic) Exchanges and Reversed-phase (or Hydrophobicity) are related to physico-chemical properties of the fixed phase. --

Please replace the paragraph at page 9, line 19 to page 10, line 5, with the following rewritten paragraph:

-- Inventors have made intensive and extensive studies on isolation of novel scorpion peptides from the venom gland of a scorpion *Tityus serrulatus*, on the basis of their ability to show any biological activity. As a result, the inventors have succeeded in isolation and purification of a novel peptide family, named Scorpion Hypotensive Peptides (SHptP), represented by SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4 (~~as described in claim 1 and claim 2~~), in which anti-hypertensive properties were found. They have also succeeded in determination of the primary and higher-order structures of TsHpt-I (SEQ ID NO: 1). It has been found that native and synthetic TsHpt-I (SEQ ID NO: 1) exhibits strong ability to decrease blood pressure in rats. Thus, the invention has been accomplished. --.

Please replace the paragraph at page 10, lines 6-11, with the following rewritten paragraph:

-- This invention ~~are~~is related to a family of peptides, which will be ~~refereed~~referred to as Scorpion Hypotensive Peptides (SHptP), found in scorpion venom, which have common structural and pharmacological features. The structural and pharmacological features that can be used in this peptides family identification are listed: --.

Please replace the paragraph at page 11, lines 1-4, with the following rewritten

paragraph:

-- Animals were collected in the Minas Gerais State, Brazil, and their venom ~~were~~was collected by electrical stimulus. Alternatively, manual stimulus can also be applied with the same result. --.

Please replace the paragraph at page 11 line 17 to page 12, line 8, with the following rewritten paragraph:

-- Purified peptides, as judged by reversed phase HPLC profile and mass spectrometry analyses were submitted to further investigation in order to determine their primary structure. Two main molecular species, measuring 2652.25 Da and 2724.64 Da, were identified by MALDI-TOF mass spectrometry analyses and subjected to amino acid hydrolysis and subsequent analysis, and amino acid sequencing by automated Edman's degradation. The sum of these analyses, i.e., HPLC chromatographic profiles, MALDI-TOF mass spectrometry, amino acid hydrolysis and analysis and amino acid sequencing by Edman's degradation, lead~~ed~~ us to identify four isoforms molecules belonging to the same structural family that were named: TsHpt-I (*Tityus serrulatus* Hypotensin-I), TsHpt-II (*Tityus serrulatus* Hypotensin-II), TsHpt-III (*Tityus serrulatus* Hypotensin-III) and TsHpt-IV (*Tityus serrulatus* Hypotensin-IV), identified by the referred sequences SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4 (as ~~described in claim 1 and claim 2~~). --

Please replace the paragraph at page 12, lines 9-22, with the following rewritten paragraph:

-- All of the four molecules described here are linear polypeptide chains without any Cys

residues and, therefore, without any internal disulfide bridge. TsHpt-I and TsHpt-II are composed ~~by~~of 25 amino acid residues, being the sole difference between these two chains, the amino acid in position 15, which is a Gln residue in TsHpt-I and a Glu residue in TsHpt-II. The other two molecules found, i.e., TsHpt-III and TsHpt-IV are composed ~~by~~of 24 amino acid residues differing from TsHpt-I and TsHpt-II by the absence of the C-terminal residue Ala. TsHpt-III and TsHpt-IV differ one from the other by the same mutation that was observed for TsHpt-I and TsHpt-II, i.e., a shift in the residue at position 15, which is a Gln in TsHpt-III sequence and a Glu in the TsHpt-IV sequence. --.

Please replace the paragraph at page 12, line 23 to page 13, line 2, with the following rewritten paragraph:

-- Peptides of the present invention, belonging to the Scorpion Hypotensive Peptides (SHptP) family, can be isolated from scorpion venoms. They may also be synthesized, chemically or by recombinant techniques from the isolated gene encoding the peptide or its preprotein or from a synthetic or cDNA copy of the gene. --.

Please replace the paragraph at page 13, lines 13-18, with the following rewritten paragraph:

-- Nucleotides sequences encoding peptides or variants of the peptides of the present invention are also within the scope of this invention. Insect viruses and plants may be engineered to express the peptides and/or variants in a manner to render themselves as vectors, vehicles or excipients for the peptides to another organism. --.

Please replace the paragraphs at page 13, line 19 to page 14, line 20, with the following rewritten paragraphs:

-- It is well-known that there is substantial redundancy in the various codons which code for specific amino acids. Therefore, this invention is also directed to those DNA sequences which contain alternative codons which code the identical amino acid. For purposes of this specification, a sequence ~~beating~~having one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are variations in the DNA sequence which do not alter the ultimate physical properties of the expressed protein. (US Patent 5,494,895, Garcia, et al., 27 February 1996).

This invention also concerns chemical modification of the peptides of the present invention. Although native peptides described by this invention did not show any post-translational modification as, for instance, acetylation, deamidation, pyroglutamic acid formed from Gln, C-terminal amide formed from Gly, methylation, phosphorylation, and others, such modifications of the native, synthesized or recombinant peptides may be achieved. These modifications may be important to protect peptides from ~~enzimatic~~enzymatic degradation leading to an extended half-life of peptides used as drugs.

This invention also concerns ~~to the~~ anti-hypertensive properties of Scorpion Hypotensive Peptides (SHptP) family and the application of peptides belonging to SHptP family as hypotensive agents in pharmaceutical applications for hypertension treatments and purposes.

TsHpt-I were found to be able to potentiate bradykinin effects in a strong and long-~~lasted~~lasting manner. --

Please replace the paragraph at page 16, lines 5-18, with the following rewritten paragraph:

-- Further purification of peptides is carried out preferably on HPLC system with a reverse-phase C4 or C18 column and eluted by a gradient using a non-polar solvent. Mobile phases (solutions) are preferably: A) Trifluoroacetic acid (TFA) 0.1 %, v/v, in pure grade ~~water~~water and B) TFA 0.1 %, v/v in Acetonitrile. Lyophilized samples are dissolved in solution A and load onto column (RP-C18) using a solution A flow at 1mL/minute, preferably. Gradient is achieved by introducing solution B, preferably at 0.01 to 1 % by minute. Elution profile is monitored by absorbance at 210-230 nm. Proteins eluted can also be monitored by mass spectrometry analyses. Alternatively ionic changes columns can be used using any liquid chromatography system. --.

Please replace the paragraph at page 17, lines 6-17, with the following rewritten paragraph:

-- After isolation from semi-purified fractions, two samples containing: i) a peptide with an observed molecular mass of 2652.25 Da and ii) a peptide with an observed molecular mass of 2724.64 Da were submitted to amino acid analysis and sequencing as described: Lyophilized samples (1 nmol) were submitted to acidic hydrolysis under N_2 atmosphere at 110° C by 24 or 72 hours using a Pico-TagTM work-station (Millipore/Waters Associates). After hydrolysis, samples were analyzed on a Beckman 6300 apparatus. To amino acid sequencing, native peptides (1 nmol) were submitted to Edman automated sequencing using an Applied Biosystems 476A sequencer. --.

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Please replace the paragraph at page 22, lines 19-21, with the following rewritten paragraph:

-- Accordingly, the scope of the invention should be determined not by the embodiments illustrated, but by the ~~apended~~appended claims and their legal equivalents. --